

PATENT COOPERATION TREATY

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NOTIFICATION OF ELECTION
(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

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ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 11 December 1998 (11.12.98)	To: United States Patent and Trademark Office (Box PCT) Crystal Plaza 2 Washington, DC 20231 ÉTATS-UNIS D'AMÉRIQUE in its capacity as elected Office
International application No. PCT/FI98/00418	Applicant's or agent's file reference peptides
International filing date (day/month/year) 18 May 1998 (18.05.98)	Priority date (day/month/year) 19 May 1997 (19.05.97)
Applicant ZAVIALOV, Vladimir Petrovich et al	

1. The designated Office is hereby notified of its election made:

in the demand filed with the International Preliminary Examining Authority on:

16 November 1998 (16.11.98)

in a notice effecting later election filed with the International Bureau on:

2. The election was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Athina Nickitas-Etienne Telephone No.: (41-22) 338.83.38
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PATENT COOPERATION TREATY
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Article 36 and Rule 70)

Applicant's or agent's file reference peptides	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/FI98/00418	International filing date (day/month/year) 18.05.1998	Priority date (day/month/year) 19.05.1997
International Patent Classification (IPC) or national classification and IPC6 A 61 K 38/04, A 61 K 38/13, A 61 K 38/21		
Applicant Zavialov, Vladimir Petrovich et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

- I Basis of the report
- II Priority
- III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV Lack of unity of invention
- V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI Certain documents cited
- VII Certain defects in the international application
- VIII Certain observations on the international application

Date of submission of the demand 16.11.1998	Date of completion of this report 01.09.1999	
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. 08-667 72 88	Telex 17978 PATOREG-9	Authorized officer Carolina Palmcrantz Telephone No. 08-782 25 00

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/FI98/00418

I Basis of the report

1. This report has been drawn on the basis of (Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.):

the international application as originally filed.

the description, pages 1-13, as originally filed,

pages _____, filed with the demand,

pages _____, filed with the letter of _____

pages _____, filed with the letter of _____

the claims, Nos. _____, as originally filed,

Nos. _____, as amended under Article 19,

Nos. _____, filed with the demand,

Nos. 1-4, filed with the letter of 31.08.1999,

Nos. _____, filed with the letter of _____

the drawings, sheets/fig 1-6, as originally filed,

sheets/fig _____, filed with the demand

sheets/fig _____, filed with the letter of _____

sheets/fig _____, filed with the letter of _____

2. The amendments have resulted in the cancellation of:

the description, pages _____

the claims, Nos. _____

the drawings, sheets/fig _____

3. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the supplemental Box (Rule 70.2(c)).

4. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/FI98/00418

V. Resoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-4	YES
	Claims		NO
Inventive step (IS)	Claims	1-4	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-4	YES
	Claims		NO

2. Citations and explanations

Amended claims 1-4 were filed 31.08.1999 wherein the wording "related" of claim 1 has been amended to "corresponding" in order to define the intended bioactive peptides in a clear and concise manner.

The present application pertains to a drug composition consisting of immunosuppressants cyclosporins, FK506, or rapamycin and the bioactive peptides corresponding to the high-affinity binding/antilymphoproliferative site of interferons $\alpha, \beta, \omega, \tau$ (e.g. alpha-peptoferon), or recombinant proteins carrying one or more of the sequences corresponding to the structures of the bioactive peptides corresponding to the high affinity-binding/antilymphoproliferative site of the interferons. The aim of the present invention is considered to be to amplify the immunosuppressants' activities so that it is possible to decrease their therapeutic dose, thereby, avoiding undesirable side effects.

The International Search Report revealed five documents of interest:

A) Dialog Information Services, file 155, Medline, Dialog accession no. 08309301, Medline accession no. 95272551, Zav'Yalov VP et al: "The sequence 130-137 of human interferon-alpha 2 is Involved in the competition of Interferon, prothymosin alpha and cholera toxin B subunit for common receptors on human fibroblasts"; & Mol Immunol, Apr 1995, 32 (6) p425-31

.../...

Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: V

B) Dialog Information Services, file 73, Embase, Dialog accession no. 10073054, Zarogoulidis K. et al: "Interferon alpha-2a and combined chemotherapy as first line treatment in SCLC patients: A randomised trial"; & Lung Cancer, 1996, 15/2 (197-205)

C) FR 2706772 AI (VACSYN (S.A.)), 30 December 1994 (30.12.94), claims 1 and 12

D) WO 9410313 A2 (UNIVERSITY OF FLORIDA), 11 May 1994 (11.05.94), the abstract; page 30, line 27 - page 31, line 4; page 40, lines 27-33; page 41, lines 23-30

E) WO 9009806 A2 (UNIVERSITY OF FLORIDA), 7 Sept 1990 (07.09.90), page 3, lines 15-21; page 16, lines 15-19; and claim 17

Document A discloses a synthetic peptide covering the sequence 130-137 of IFN-alpha 2 (alpha-peptoferon).

Document B pertains to a study for detection of clinical benefits of the addition of IFN-alpha in the induction of chemotherapy of SCLC patients. The addition of IFN-alpha to induce chemotherapy appears to confer a survival benefit to SCLC patients but optimal dosing schedule has yet to be defined.

Document C concerns a pharmaceutical composition comprising an immunosuppressive agent, e.g., ciclosporine A or FK506, and IFN-alpha (see claims 1 and 12).

Document D concerns interferon tau compositions. It is suggested in the document that the IFN-tau (119-150) region is primarily responsible for inhibition of cell growth (see page 30, lines 27-33). The composition could be used as an immunosuppressant to prevent interferon-mediated immune responses to tissue transplants (see page 41, lines 23-30).

.../...

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of:

Document E relates to a composition, for inhibiting tumour growth or viral replication, comprising a peptide fragment of oTP-1 (oTP-1 has been found to be structurally similar to bovine interferon alpha II). The composition may also include other antiviral or tumour inhibiting substances (see page 2, lines 23-25; page 16, lines 15-19; and claim 17).

In conclusion, none of the cited documents discloses a drug composition consisting of immunosuppressants cyclosporins, FK506 or rapamycin in combination with peptides corresponding to the high-affinity binding/antilymphoproliferative site of interferons a,b,w,t. This synergistic combination is, therefore, considered to fulfil the requirements of novelty, inventive step and industrial applicability.

31-08-1999

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Claims

1. Any drug composition consisting of immunosuppressants cyclosporins, FK506, or rapamycin and the bioactive peptides corresponding to the high-affinity binding/anti-lymphoproliferative site of interferons a,b,w,t, or recombinant proteins carrying one or more of the sequences corresponding to the structures of the bioactive peptides corresponding to the high-affinity binding/anti-lymphoproliferative site of the said interferons for the aim of amplification of immunosuppressants' activities to decrease their therapeutic dose, and as the consequence to avoid their undesirable side effects during 5 organ and tissue transplantation or during treatment of cancers such as lymphomas, leukemias, myelomas, adenocarcinomas, autoimmune and chronic inflammatory diseases, such as rheumatoid arthritis, myasthenia gravis, lupus erythematosus, uveitis, 10 hyperproliferative diseases, such as psoriasis vulgaris, wherein cyclosporins, FK506 or rapamycin can be exploited.

15 2. Compositions according to Claim 1 consisting of immunosuppressants cyclosporins, FK506 or rapamycin with one or more of the peptides containing at least 5 identical amino acid residues in the following table:

L	T	E	K	K	Y	S	P
R	R	R	R	H	R	R	
Q	D	N	D		D	D	
M	L	M	N		N	L	
	I	S	S		S		
E	A	E			A		
G	I						

20

wherein,

each amino acid residue in the top sequence can be modified with any of the amino acid 25 residues under it.

3. Compositions according to Claim 1 consisting of immunosuppressants cyclosporins, FK506 or rapamycin, and recombinant proteins carrying one or more of the sequences corresponding to the peptide variation of Claim 2.

5

4. Compositions according to Claim 2 consisting of immunosuppressants cyclosporins, FK506 and rapamycin, and the bioactive peptides wherein the active peptides are genetically or chemically modified or genetically or chemically or physically bound to a small-molecular or macromolecular substance for the aim of increasing the stabilities of the 10 peptides in physiological conditions or for regulating the bioavailability of the said peptides.

Claims

1. Any drug composition consisting of immunosuppressants cyclosporins, FK506, or rapamycin and the bioactive peptides ^{corresponding} ~~related~~ to the high-affinity binding/anti-lymphoproliferative site of interferons $\alpha, \beta, \omega, \tau$ or recombinant proteins carrying one or more of the sequences corresponding to the structures of the bioactive peptides related to the high-affinity binding/anti-lymphoproliferative site of the said interferons for the aim of amplification of immunosuppressants' activities to decrease their therapeutic dose, and as the consequence to avoid their undesirable side effects during organ and tissue transplantation or during treatment of cancers such as lymphomas, leukemias, myelomas, adenocarcinomas, autoimmune and chronic inflammatory diseases, such as rheumatoid arthritis, myasthenia gravis, lupus erythematosus, uveitis, hyperproliferative diseases, such as psoriasis vulgaris, wherein cyclosporins, FK506 or rapamycin can be exploited.
15. 2. Compositions according to Claim 1 consisting of immunosuppressants cyclosporins, FK506 or rapamycin with one or more of the peptides containing at least 5 identical amino acid residues in the following table:

L	T	E	K	K	Y	S	P
R	R	R	R	H	R	R	
Q	D	N	D		D	D	
M	L	M	N		N	L	
	I	S	S		S		
E	A	E			A		
G	I						

20

wherein,

each amino acid residue in the top sequence can be modified with any of the amino acid residues under it.

3. Compositions according to Claim 1 consisting of immunosuppressants cyclosporins, FK506 or rapamycin, and recombinant proteins carrying one or more of the sequences corresponding to the peptide variation of Claim 2.

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4. Compositions according to Claim 2 consisting of immunosuppressants cyclosporins, FK506 and rapamycin, and the bioactive peptides wherein the active peptides are genetically or chemically modified or genetically or chemically or physically bound to a small-molecular or macromolecular substance for the aim of increasing the stabilities of the peptides in physiological conditions or for regulating the bioavailability of the said peptides.

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REC'D 22 SEP 1999

WIPO PCT

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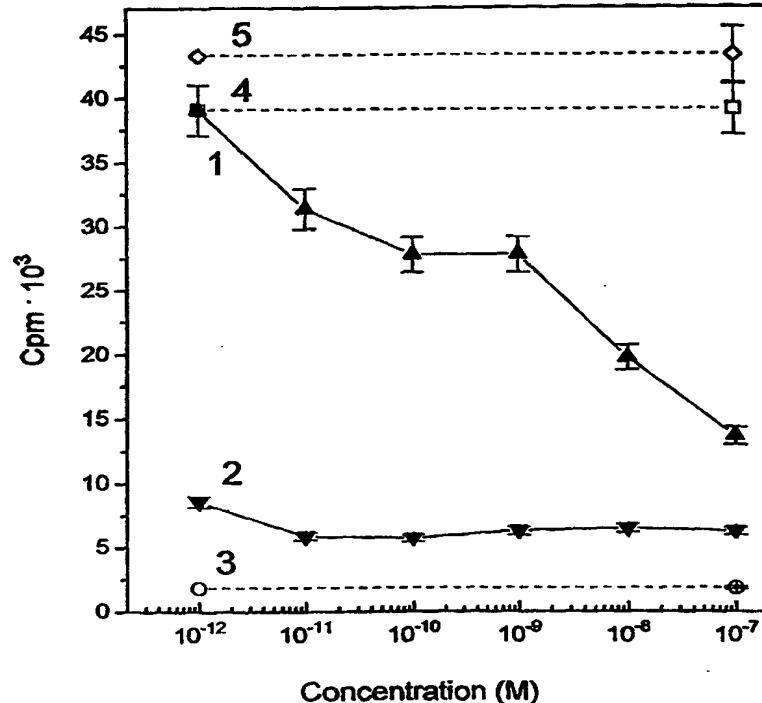
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 38/04, 38/13, 38/21	A1	(11) International Publication Number: WO 98/52594 (43) International Publication Date: 26 November 1998 (26.11.98)
(21) International Application Number: PCT/FI98/00418		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).
(22) International Filing Date: 18 May 1998 (18.05.98)		
(30) Priority Data: 972121 19 May 1997 (19.05.97) FI		
(71)(72) Applicants and Inventors: ZAVIALOV, Vladimir Petrovich [RU/RU]; Flat 12, Zavodskaya street, 10, Chekhov District, Moscow Region, Lyubuchany, 142380 (RU). VASILENKO, Raisa Nikolaevna [RU/RU]; Flat 49, Zavodskaya street, 4, Chekhov District, Moscow Region, Lyubuchany, 142380 (RU). DOLGIKH, Dmitry Aleksandrovich [RU/RU]; Flat 445, Bolshaya Serpukhovskaya street, 46, corpus 46, Moscow, 113093 (RU). KIRPICHNIKOV, Mikhail Petrovich [RU/RU]; Flat 52, Tverskaya street, 15, Moscow, 113000 (RU). NAVOLOTSKAYA, Elena Vitalievna [RU/RU]; Flat 72, Microdistrict "G", 9, Moscow Region, Pushchino, 142292 (RU). KORPELA, Timo, Kalevi [FI/FI]; Kasarminkatu 5 as. 8, FIN-20500 Turku (FI).		
(74) Common Representative: KORPELA, Timo, Kalevi; Kasarminkatu 5 as. 8, FIN-20500 Turku (FI).		

(54) Title: COMPOSITIONS FOR ENHANCING IMMUNOSUPPRESSANTS' PHARMACEUTICAL ACTIVITIES

(57) Abstract

The present invention provides the compositions for efficient amplification of immunosuppressive activity of cyclosporins, FK506 or rapamycin to decrease therapeutical dose of immunosuppressants, and as the consequence to avoid their undesirable side effects during organ and tissue transplantation, and treatment of different diseases. These compositions include cyclosporins, FK506 or rapamycin and biologically active peptides corresponding to the high-affinity binding/anti-lymphoproliferative site of IFNs- α , β , ω , τ , or recombinant proteins having the amino acid sequences corresponding to the said site.



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COMPOSITIONS FOR ENHANCING IMMUNOSUPPRESSANTS, PHARMACEUTICAL ACTIVITIES

5 FIELD OF INVENTION

This invention is related to human or animal medicine and more specifically to improving the therapeutic effects of common immunosuppressive, anti-inflammatory and/or antiparasitic drugs where the effective ingredients contain cyclosporins, FK506 or 10 rapamycin. This invention provides new compositions for efficient amplification of the activities of the said components to decrease their therapeutic dose to avoid their side effects.

BACKGROUND OF INVENTION

15 Cyclosporins are cyclic peptides composed of eleven amino acid residues which include previously unknown N-methylated amino acid residues. Cyclosporins are commercially important products isolated from the culture broths of the fungal species *Tolypocladium*. Cyclosporins are applied to prolong survival of allogenic transplants involving skin, 20 kidney, pancreas, bone marrow, small intestine and lung. The efficacy of cyclosporins is presently known to be due to the specific and reversible inhibition of immunocompetent cells, primarily T-helper cells. Unfortunately, cyclosporins' systemic use have been associated with a high incidence of renal toxicity (kidney failure), some cases of hepatotoxicity, increased incidence of lymphoid tumors and increased incidence of 25 opportunistic infections. The side effects of cyclosporins are so severe and so common that they limit their applications to life-threatening or severe sight-threatening diseases.

Cyclosporins, like FK506 and other related molecules, bind specifically to certain physiologically important molecular species in tissues and blood circulation (Denesyuk A. 30 et al., 1993, Biochem. Biophys. Res. Commun. 197, pp. 1438-1442; Denesyuk A. et al., 1993, Biochem. Biophys. Res. Commun. 194, pp. 280-286; Denesyuk A. et al., 1993, Biochem. Biophys. Res. Commun. 192, pp. 912-917). After identification of cyclosporin

A, other cyclosporins, B, C, D, E, and G, containing minor differences in the molecular structure of cyclosporins have been reported. Many of these cyclosporins exhibit pharmaceutical utility related to cyclosporin A and the literature involves abundantly examples and suggestions for their use as therapeutic agents.

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Cyclosporins were first proposed for be used as an antifungal agents, but their immunosuppressive action was found to be of more importance. Cyclosporins specifically and reversibly inhibit immunocompetent cells, primarily T-helper cells, by specific interactions with peptidyl-prolyl *cis-trans* isomerase (PPIase; see, Schreiber S. and 10 Crabtree G., 1992, *Immunol. Today* 13, pp. 136-142; Zav'yalov V. et al., 1995, *Acta Pathol. Microbiol. Immunol. Scand.* 103, pp. 401-415). These multifunctional PPIase proteins may also serve as ATP-independent molecular chaperones facilitating the folding 15 of target proteins and assembly of multisubunit protein complexes (Schmid F. et al., 1993, *Adv. Protein Chem.* 46, pp. 25-65), but their primary function is the binding of the immunosuppressive drugs cyclosporin A, FK506 and rapamycin. The PPIases with such activity were termed immunophilins (ImP's). These protein receptors fall into two major groups for their structure: the cyclosporin binding proteins, also termed cyclophilins (CyPs), and the FK506 binding proteins, termed FKBP's, some of the latter preferentially binding rapamycin (Trandini C. et al., 1992, *FASEB J.* 6, pp. 3410-3420; Liu J., 1993, 20 *Immunol. Today* 14, pp. 290-295). It has been found that Ca^{2+} and calmodulin-dependent protein phosphatase, calcineurin (CaN), binds with high affinity to biologically active ImP-drug complexes. The experimental data suggest that the cytoplasmic NF-AT protein (NF-ATc) is a substrate, either direct or indirect, via a phosphatase cascade for CaN. NF-AT is a complex protein made of at least two subunits. One subunit of NF-AT (NF-ATp), 25 a DNA-binding phosphoprotein, is a substrate for CaN *in vitro* and interacts with Fos and Jun proteins in the nucleus of activated T cells (Jain J. et al., 1993, *Nature* 365, pp. 352-355). Dephosphorylation by CaN might directly induce nuclear translocation of NF-ATc. The active form of this protein interacts with functional sequences within the IL-2 enhancer. According to a hypothesis, the complexes cyclosporin A-CyP or FK506-FKBP- 30 12 block nuclear translocation of NF-ATc and activation of IL-2 gene expression due to the inhibition of CaN activity.

Cyclosporins' immunosuppressive properties have led to their wide routine usage in immune system-related diseases, as described, for example, in Finnish patent publication 900604. Correspondingly, the number of patents for these purposes on various aspects of cyclosporins is large. In addition, eye medicine has benefited from cyclosporins. The U.S.

5 Patent No. 4, 649, 047 describes a method for the treatment of phacoanaphylactic endophthalmitis and uveitis in the anterior or posterior segment of an eye wherein cyclosporin is topically administered to the eye. In other ophthalmic applications, cyclosporin has been used topically only for the treatment of external (e.g., corneal) eye diseases. BenEzra et al., 1986, Amer. J. Ophthalmol. 101, pp. 278-282 described the effect
10 of 2% cyclosporin eyedrops on severe vernal keratoconjunctivitis which is a seasonal allergic disorder unrelated to tear deficiency. Hunter et al., 1981, Clin. Exp. Immunol. 45, pp. 173-177 described the topical administration of cyclosporin in a rabbit model of corneal graft rejection with positive results. Boisjoly et al., 1984, Arch. Ophthalmol. 102, pp. 1804-1807 reported that topical application of cyclosporin had a beneficial
15 prophylactic effect towards the treatment of severe herpetic stromal keratitis. Mosteller et al., 1984, Investigative Ophthalmol. Supp. 25, pp. 3-38 treated corneal allograft rejection in rabbits by applying a single dose of a 10% cyclosporin A ointment in the lower cul-de-sac of the eyelids. Cyclosporins have also been used systemically in other ophthalmic applications, where the disease being treated is not limited to the eye surface. For
20 example, Nussenblatt et al., 1983, Amer. J. Ophthalmol. 96, pp. 275-282 reported clinical improvement in some patients with noninfectious posterior uveitis following systemic treatment with cyclosporin.

25 Cyclosporins are primarily administered orally or by injection. Unfortunately, cyclosporins used systemically have been associated with a high incidence of renal toxicity (kidney failure), some cases of hepatotoxicity, increased incidence of lymphoid tumors and increased incidence of opportunistic infections. Cyclosporin are only slightly less toxic than other immunosuppressive agents such as cytoxan or azathioprine. The systemic side effects of cyclosporins are so severe and so common that they limit
30 cyclosporins use to life-threatening or in some cases severe sight-threatening diseases.

As described in U.S. Patent No. 4, 117, 118 1978 (Harri et al.), cyclosporin is readily soluble in most of the usual organic solvents and practically insoluble in petroleum ether and water. Poor solubility of cyclosporins makes absorption in intestine with oral administration as well as actions in intravenous applications variable from patient to 5 patient. If rather high and constant level of cyclosporins are required in the blood circulation, administration of cyclosporins is complex. As distributed by Sandoz Ltd. under the tradename Sandimmune, cyclosporin for oral administration is dissolved in olive oil for further dilution with food and in polyoxyethylated castor oil and ethanol for intravenous injection. The release of cyclosporins from the carrier substances is patient- 10 dependent where either the dose is not therapeutically effective or it is toxic. As stated, prolonged systemic administration of cyclosporins associated with transplantation and related uses creates risks for other diseases including cancers. To be able to use considerably lower than toxic level of effective cyclosporin doses is therefore of key importance.

15

Recently, the attention was brought to the long-neglected role of type I interferons (IFN-a,b,w,t) in the modulation of immune response (Belardelli F. and Gresser I., 1996, Immunol. Today 17, pp. 369-372). The immunomodulating activity of IFN-a,b,w,t is very complex and includes:

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- inhibition of lymphocyte proliferation after stimulation with lectins or allogenic cells;
- prolongation of skin grafts;
- inhibition of delayed type of hypersensitivity;
- differentiation towards a Th1 type of immune response;
- 25 -stimulation *in vivo* of long-lived antigen-specific memory CD8+ cell cytotoxicity;
- inhibition of suppressor T cells;
- enhancement of natural killer (NK) cell cytotoxicity;
- enhancement of major histocompatibility complex (MHC) class I antigens expression.

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First three immunomodulating properties of type I IFNs (inhibition of lymphocyte proliferation after stimulation with lectins or allogenic cells, prolongation of skin grafts, and inhibition of delayed type of hypersensitivity) are useful for amplification of

immunosuppressants' (cyclosporins, FK506 and rapamycin) activity. But other immunomodulating properties of type I IFNs (differentiation towards a Th1 type of immune response; stimulation *in vivo* of long-lived antigen-specific memory CD8⁺ cell cytotoxicity; inhibition of suppressor T cells; enhancement of NK cell cytotoxicity; 5 enhancement of MHC class I antigens expression) can induce an opposite effect. Indeed, it was observed that a monoclonal antibody directed against the extracellular domain of the human (h)IFN- α receptor, which inhibits both the binding and biological activity of all the type I IFNs tested, exerted a dose-dependent inhibition of the mixed lymphocyte reaction and induced permanent survival of skin allografts in MHC-divergent 10 Cynomologus monkeys treated with a subeffective dose of cyclosporin A (Tovey, M.G. et al, 1996, *J. Leukocyte Biol.* 59, pp. 512-517).

In the present invention we surprisingly observed that the physiological effects of immunosuppressants (cyclosporins, FK506 and rapamycin) can be dramatically increased 15 by the simultaneous administration of any one of the immunosuppressants and the peptide corresponding to the high-affinity binding/anti-lymphoproliferative site of IFN- α or the recombinant protein having the amino acid sequence corresponding to the said site. Peptides are specially favourable additives since they do not cause harmful symptoms and the activity quenching due to the generation of antibodies against larger polypeptides 20 such as natural interferons.

Long time ago there was discovered the primary structure homology of hIFNs- α / β and thymus hormone - thymosin α_1 (TMA₁) (Zav'yalov V. and Denesyuk A., 1984, *Doklady Akademii Nauk SSSR* 275, pp. 242-246). Comparison of the primary structures of 25 proTMA and hIFNs- α / β reveals a homology of the prohormone with the IFNs' sites making up the C-terminal helices D and E, however, the highest homology is observed for the C-terminal part of helix D and the N-terminal part of loop DE (Zav'yalov V. et al., 1989, *Immun. Lett.* 22, pp. 173-182; Zav'yalov V. et al., 1990, *Biochim. Biophys. Acta* 1041, pp.178-185). One of the synthesized peptides (α -peptoferon) overlapping the 30 TMA₁-like sequence of hIFN- α 2, effectively competed with hIFN- α 2, TMA₁, proTMA for common receptors on mouse thymocytes (i.e. the K_i of recombinant (r)hIFN- α 2 binding by α -peptoferon is equal to about 10⁻¹² M) (Zav'yalov V. et al., 1991, *FEBS Lett.* 278,

pp. 187-189; Zav'yalov V. et al., 1995, Molec. Immun. 32, pp. 425-431). Recently the first example of successful grafting of hIFN-a2's TM_{a1}-like site to the design *de novo* protein albeferon was described (Dolgikh D. et al., 1996, Protein Engin. 9, pp. 195-201). The IFN-a2 fragment corresponded to the TM_{a1}-like sequence 130-137 was inserted into 5 the N-termini of an albebetin molecule just after the initiatory Met residue. The chimeric protein (albeferon) was expressed in a wheat germ cell-free translation system and tested for its compactness and stability. It has been shown that albeferon is practically as compact as natural proteins of corresponding molecular weight and possesses high 10 stability toward the urea-induced unfolding. To testify the affinity of albeferon to murine thymocyte receptor, the protein inhibitory effect on the binding of radiolabeled [¹²⁵I] a-peptoferon to the receptors has been studied. The albeferon competitive inhibition coefficient (IC₅₀) and the calculated inhibition constant (K_i) are very close to that of rhIFN-a2. It was demonstrated that cell surface binding characteristics correlate with 15 consensus type I IFN enhanced anti-lymphoproliferative activity on the human periferal polymorphonuclear cells (Klein S. et al., 1996, J. Interferon and Cytokine Res. 16, pp. 1-6; Dhib-Jalbut S. et al., 1996, J. Interferon and Cytokine Res. 16, pp. 195-200).

The present invention includes the compositions for efficient amplification of 20 immunosuppressive activity of cyclosporins, FK506 or rapamycin by peptides corresponding to the high-affinity binding/anti-lymphoproliferative site of IFNs-a,b,w,t or recombinant proteins having the amino acid sequences corresponding to the said site to decrease therapeutic dose of the both groups of compounds, and as the consequence to avoid their undesirable side effects during organ and tissue transplantation, and treatment 25 of different diseases.

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SUMMARY OF THE INVENTION

The present invention provides the compositions for efficient amplification of 30 immunosuppressive activity of cyclosporins, FK506 or rapamycin to decrease therapeutical dose of immunosuppressants, and as the consequence to avoid their

undesirable side effects during organ and tissue transplantation, and treatment of different diseases.

These compositions include cyclosporins, FK506 or rapamycin and biologically active peptides corresponding to the high-affinity binding/anti-lymphoproliferative site of IFNs-a,b,w,t, or recombinant proteins having the amino acid sequences corresponding to the said site.

BRIEF DESCRIPTION OF THE DRAWINGS

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FIG. 1 Effect of rhIFN-a2 and of the peptide corresponding to the high-affinity binding/anti-lymphoproliferative site of hIFN-a2 (a-peptoferon) on mitogen (PHA)-driven proliferation of human peripheral polymorphonuclear cells.

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FIG. 2 Effect of rhIFN-a2 and of the peptide corresponding to the high-affinity binding/anti-lymphoproliferative site of hIFN-a2 (a-peptoferon) on the proliferation of an IFN-sensitive human T-lymphoblastoid cell line MT-4.

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FIG. 3 Effect of rhIFN-a2, albeferon and albebetin on mitogen (PHA)-driven proliferation of human peripheral polymorphonuclear cells.

FIG. 4 Effect of rhIFN-a2, albeferon and albebetin on the proliferation of an IFN-sensitive human T-lymphoblastoid cell line MT-4.

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FIG. 5 Effect of rhIFN-a2 and of the peptide corresponding to the high-affinity binding/anti-lymphoproliferative site of hIFN-a2 (a-peptoferon) on mitogen (PHA)-driven proliferation of human peripheral polymorphonuclear cells in the presence of 20 mM cyclosporin A in cultural medium.

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FIG. 6 Effect of albeferon on mitogen (PHA)-driven proliferation of human peripheral polymorphonuclear cells in the presence of 10 mM cyclosporin A in cultural medium.

DETAILED DESCRIPTION OF THE INVENTION

Cyclosporins, FK506 and rapamycin are widely used commercially important drugs for treatment of various diseases of human and higher animals. However, the use of these 5 drugs have severe drawbacks for their toxicity and narrow effective dose range. This forms the basis of either developing other more effective and less toxic related drugs or increasing their activity by other ingredients. The latter has been attempted only by making drug formulations which provide either more effective absorption in intestine or prolonged delivery in intestine or in intravenous administration.

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In this invention, there was surprisingly observed that certain favourable physiological effects of cyclosporins, FK506 and rapamycin on human cells can be amplified by biologically active peptides corresponding to the high-affinity binding/anti-lymphoproliferative site of IFNs-a,b,w,t or recombinant proteins having the amino acid sequences corresponding to the said site. The peptides and recombinant proteins mentioned above can be readily produced by widely available synthetic or recombinant techniques. The present invention provides definite improvement of compositions for drugs based on cyclosporins, FK506 or rapamycin aimed for human and higher animals. 15 These compositions include the immunomodulators and biologically active peptides corresponding to the high-affinity binding/anti-proliferative site of IFNs-a,b,w,t, or recombinant proteins having the amino acid sequences corresponding to the said site. Although all the potentially bioactive peptides or the recombinant proteins containing the amino acid sequences of the peptides were not possible to test experimentally here with the immunomodulator drugs (cyclosporins, FK506 and rapamycin) such structures were 20 revealed by extensive comparisons of available amino acid sequences of interferons from different species by computer and molecular modelling techniques taking into account the experimental data on the localisation of the high-affinity binding/anti-lymphoproliferative site of IFNs-a,b,w,t and the experimental data on the competition of type I IFNs for the common receptors. The peptides according to this invention have the advantage of not 25 give rise to antibodies like large polypeptides and natural interferons and thus peptides as such or bound to carrier molecules can be used for long periods.

In the previous studies (Zav'yalov V. et al., 1991, FEBS Lett. 278, pp. 187-189; Zav'yalov V. et al., 1995, Molec. Immun. 32, pp. 425-431) it was demonstrated that only octapeptide corresponding to the 130-137 amino acid residues of hIFN- α 2 had the same or higher affinity to the specific receptors in comparison with recombinant hIFN- α 2. It is 5 well-known that all type I IFNs compete for the common receptors and can induce the common type I IFN activities. Therefore, it is reasonable to assume that in the process of natural selection the changes in the amino acid sequence of the high-affinity binding/anti-lymphoproliferative site of IFNs-a,b,w,t were selected not to abolish the biological activities of the site. Consequently, all type I natural and recombinant IFNs as well as 10 peptides corresponding to their high-affinity binding/anti-lymphoproliferative site, and recombinant proteins having the amino acid sequences corresponding to the said site might reproduce the anti-proliferative activity and amplify immunosuppressive activity of cyclosporins.

For the testing of synergism of cyclosporin, FK506 or rapamycin and peptides 15 corresponding to the high-affinity binding/anti-lymphoproliferative site of IFNs-a,b,w,t or recombinant proteins having the amino acid sequences corresponding to the said site we employed the classic anti-lymphoproliferative test system with human peripheral polymorphonuclear cells and human T-lymphoblastoid cell line MT-4. The cell culture conditions and the conditions of the humoral cells in blood circulation are closely related. 20 In fact, in cell cultures, which are commonly used for testing of potential drugs, the conditions are strictly maintained similar to the blood circulation as to the temperature, pH, buffer, minerals, CO₂ and O₂ partial pressures and so on. On the other hand, in this special case the target cells of cyclosporins or the other immunosuppressants and the peptides corresponding to the high-affinity binding/anti-lymphoproliferative site of IFNs- 25 a,b,w,t or recombinant proteins having the amino acid sequences corresponding to the said site being used as the drugs exist specifically in the blood circulation in very equal conditions to the cell cultures. Thus, it is highly predictable that the drug compositions of the present invention can be used as medical drugs for purposes previously used for immunosuppressants alone as the active ingredient in the drug formulations.

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While the main experimental proof of the present invention lies on the use of peptides corresponding to the high-affinity binding/anti-lymphoproliferative site of IFNs-a,b,w,t or

recombinant proteins having the amino acid sequences corresponding to the said site with cyclosporins, also FK506 and rapamycin can be applied with related amplification effect as with cyclosporins. Although the present invention describes effects of peptides corresponding to the high-affinity binding/anti-lymphoproliferative site of IFNs-a,b,w,t or 5 recombinant proteins having the amino acid sequences corresponding to the said site on immunosuppressants such as cyclosporins, FK506 and rapamycin, it is evident that the peptides corresponding to the high-affinity binding/anti-lymphoproliferative site of IFNs-a,b,w,t or recombinant proteins having the amino acid sequences corresponding to the said site will increase equally well the activities of any other immunosuppressant.

10

Example 1.

Ninety five ml of blood were taken from each of 10 volunteer donors, and 5 ml of 3-5% 15 EDTA were added to the blood immediately and mixed carefully to prevent coagulation. The blood was then mixed with the equal volume of medium 199 or with buffered physiological salt solution. Ficoll-Paque (Pharmacia) was poured into plastic centrifuge test-tubes with caps (Costar) (3 ml of Ficoll-Paque per each test-tube, volume of a test-tube - 15 ml, diameter - 13 mm at room temperature, and then 6-7 ml of diluted blood 20 were carefully layered on top). The test-tubes were centrifuged at 20 °C for 40 minutes, at 400 g, with the brake of centrifuge being switched off. The mononuclear cells formed a layer on the border of the liquid division and the cells were collected with a Pasteur pipette into a centrifuge test-tube with 5 ml of RPMI 1640, containing 2% of inactivated fetal serum and penicillin (100 units/ml) with streptomycin (100 units/ml). Then the cells 25 were washed with physiological buffer solution (PBS) three times with centrifuging at 400 g for 5 minutes at the temperature of +8 °C. When the concentration of the cells was $2.5 \cdot 10^6$ cells/ml, they were placed in RPMI 1640 medium, containing 5% of fetal serum (heated for 30 minutes at 56 °C), 100 units/ml of penicillin, 100 units/ml of streptomycin, 1 ml 200 mM of L-glutamine per 100 ml of serum. All the reagents were produced by 30 Gibco. The cells were dropped into a 96-well plate (100 ml per each plate) with mitogen - phytohemagglutinin (PHA) (final concentration - 2 mg/ml), the preparations under study and immunosuppressants (cyclosporin A, FK506 or rapamycin) in concentration - 5-20

mM) were pipetted into the tubes beforehand. The plate was placed in a CO₂-incubator at 5% CO₂ with humid atmosphere for 72 hours. 12-18 hours before the completion of the incubation 1 mCi of [³H]-thymidine was added into each well. Then the cells were washed on a semiautomatic harvester (Titertec Flow Lab.) using filters (Whatman). Each filter was carefully transferred into bottles, containing 5 ml of scintillation liquid (Sigma). The number of disintegrations per minute was counted using a Minibeta counter (LKB). The stimulation index was determined by arithmetical mean of at least three parallel measurements \pm SD to the corresponding control.

10 Fig. 1 displays, respectively, the effect of rhIFN-a2 and of the peptide corresponding to the high-affinity binding/anti-lymphoproliferative site of hIFN-a2 (a-peptoferon) on mitogen (PHA)-driven proliferation of human peripheral polymorphonuclear cells. PHA induced strong lymphoproliferative response that was inhibited by rhIFN-a2 and a-peptoferon in a dose-dependent fashion. rhIFN-a2 and a-peptoferon demonstrated comparable effects. rhIFN-g had either no effect or a minimal enhancing effect on the lymphoproliferative response. (1) rhIFN-a2; (2) a-peptoferon; (3) control: PHA; (4) control: cultural medium.

Example 2.

20 Fig. 2 shows the effect of rhIFN-a2 and of the peptide corresponding to the high-affinity binding/anti-lymphoproliferative site of hIFN-a2 (a-peptoferon) on the proliferation of an IFN-sensitive human T-lymphoblastoid cell line MT-4. The proliferation of human T-lymphoblastoid cell line MT-4 was inhibited by rhIFN-a2 and a-peptoferon in a dose-dependent fashion. rhIFN-a2 and a-peptoferon demonstrated comparable effects. Similar 25 methods as in Example 1 were employed. (1) rhIFN-a2; (2) a-peptoferon.

Example 3.

Fig. 3 displays the effect of rhIFN-a2 and the designed *de novo* chimeric protein albeferon containing the TM_{a1}-like sequence 130-137 of the hIFN-a2 inserted into the N-termini of 30 the protein just after the initiatory Met residue (2), and the designed *de novo* protein albebetin without the hIFN-a2 fragment (3) on PHA-driven proliferation of human peripheral polymorphonuclear cells. PHA induced strong lymphoproliferative response

that was inhibited by rhIFN-a2 and albeferon in a dose-dependent fashion. rhIFN-a2 and albeferon demonstrated comparable effects. Albebetin had no effect on the lymphoproliferative response. The results show that the hIFN-a2 fragment corresponded to the TMa₁-like sequence 130-137 is responsible for the anti-lymphoproliferative effect 5 of rhIFN-a2. Similar methods as in Example 1 were employed. (1) rhIFN-a2; (2) albeferon; (3) albebetin; (4) control: cultural medium; (5) control: PHA

Example 4.

Fig. 4 demonstrates the effect of rhIFN-a2, albeferon and albebetin on the proliferation of 10 an IFN-sensitive human T-lymphoblastoid cell line MT-4. The proliferation of human T-lymphoblastoid cell line MT-4 was inhibited by rhIFN-a2 and albeferon in a dose-dependent fashion. In this experiment albeferon had higher activity than the sample of rhIFN-a2 used but comparable with other data on rhIFN-a2 (see Figs. 1-2 and the data described in: Dhib-Jalbut S. et al., 1996, J. Interferon and Cytokine Res. 16, pp. 195-15 200). Albebetin had no effect on the lymphoproliferative response. The results show that the hIFN-a2 fragment corresponded to the TMa₁-like sequence 130-137 is responsible for the anti-lymphoproliferative effect of rhIFN-a2. (1) rhIFN-a2; (2) albeferon; (3) albebetin.

Example 5.

20 Fig. 5 displays the effect of rhIFN-a2 on PHA-driven proliferation of human peripheral polymorphonuclear cells in the presence of 20 mM cyclosporin A (CsA) in cultural medium. PHA induced strong lymphoproliferative response. The selected concentration of CsA had enhancing effect on the lymphoproliferative response. The administration of extremely low amount of rhIFN-a2 (10^{-16} M) totally abolished lymphoproliferative 25 response induced by PHA. The comparable effect on mitogen-driven proliferation of peripheral blood human T-lymphocytes in the presence of 20 mM CsA in cultural medium was observed for the octapeptide corresponding to the high-affinity binding/anti-lymphoproliferative site of hIFN-a2 (a-peptoferon). The results demonstrate strong synergism of anti-lymphoproliferative action of CsA and rhIFN-a2 or the peptide 30 corresponding to the high-affinity binding/anti-lymphoproliferative site of hIFN-a2 (a-peptoferon). Related results were obtained with 1-50 mM concentration of FK506 or rapamycin instead of cyclosporin A. Similar methods as in Example 1 were employed. (1)

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rhIFN- α 2 and 20 mM CsA; (2) α -peptoferon and 20 mM CsA; (3) control: cultural medium; (4) control: 20 mM CsA; (5) control: PHA; (6) control: 20 mM CsA and PHA.

Example 6.

5 Fig. 6 displays the effect of albeferon on PHA-driven proliferation of human peripheral polymorphonuclear cells in the presence of 10 mM cyclosporin A (CsA) in cultural medium. PHA induced strong lymphoproliferative response. The selected concentration of CsA had enhancing effect on the lymphoproliferative response. The administration of extremely low amount of albeferon (10^{-12} M) totally abolished lymphoproliferative
10 response induced by PHA. Related results were obtained with 1-50 mM concentration of FK506 or rapamycin instead of cyclosporin A. Fig. 6 also shows that the octapeptide corresponding to the high-affinity binding/anti-lymphoproliferative site of hIFN- α 2 (α -peptoferon) which was genetically immobilized on the macromolecular carrier (i.e. on the *de novo* protein albebetin) is biologically active. Similar methods as in Example 1 were
15 employed. (1) albeferon; (2) albeferon and 10 mM CsA; (3) control: cultural medium; (4) control: PHA; (5) control: PHA and 10 mM CsA.

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Claims

1. Any drug composition consisting of immunosuppressants cyclosporins, FK506, or rapamycin and the bioactive peptides related to the high-affinity binding/anti-lymphoproliferative site of interferons a,b,w,t or recombinant proteins carrying one or more of the sequences corresponding to the structures of the bioactive peptides related to the high-affinity binding/anti-lymphoproliferative site of the said interferons for the aim of amplification of immunosuppressants' activities to decrease their therapeutic dose, and as the consequence to avoid their undesirable side effects during organ and tissue transplantation or during treatment of cancers such as lymphomas, leukemias, myelomas, adenocarcinomas, autoimmune and chronic inflammatory diseases, such as rheumatoid arthritis, myasthenia gravis, lupus erythematosus, uveitis, hyperproliferative diseases, such as psoriasis vulgaris, wherein cyclosporins, FK506 or rapamycin can be exploited.
- 15 2. Compositions according to Claim 1 consisting of immunosuppressants cyclosporins, FK506 or rapamycin with one or more of the peptides containing at least 5 identical amino acid residues in the following table:

L	T	E	K	K	Y	S	P
R	R	R	R	H	R	R	
Q	D	N	D		D	D	
M	L	M	N		N	L	
	I	S	S		S		
E	A	E			A		
G	I						

20

wherein,

each amino acid residue in the top sequence can be modified with any of the amino acid residues under it.

3. Compositions according to Claim 1 consisting of immunosuppressants cyclosporins, FK506 or rapamycin, and recombinant proteins carrying one or more of the sequences corresponding to the peptide variation of Claim 2.

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4. Compositions according to Claim 2 consisting of immunosuppressants cyclosporins, FK506 and rapamycin, and the bioactive peptides wherein the active peptides are genetically or chemically modified or genetically or chemically or physically bound to a small-molecular or macromolecular substance for the aim of increasing the stabilities of
10 the peptides in physiological conditions or for regulating the bioavailability of the said peptides.

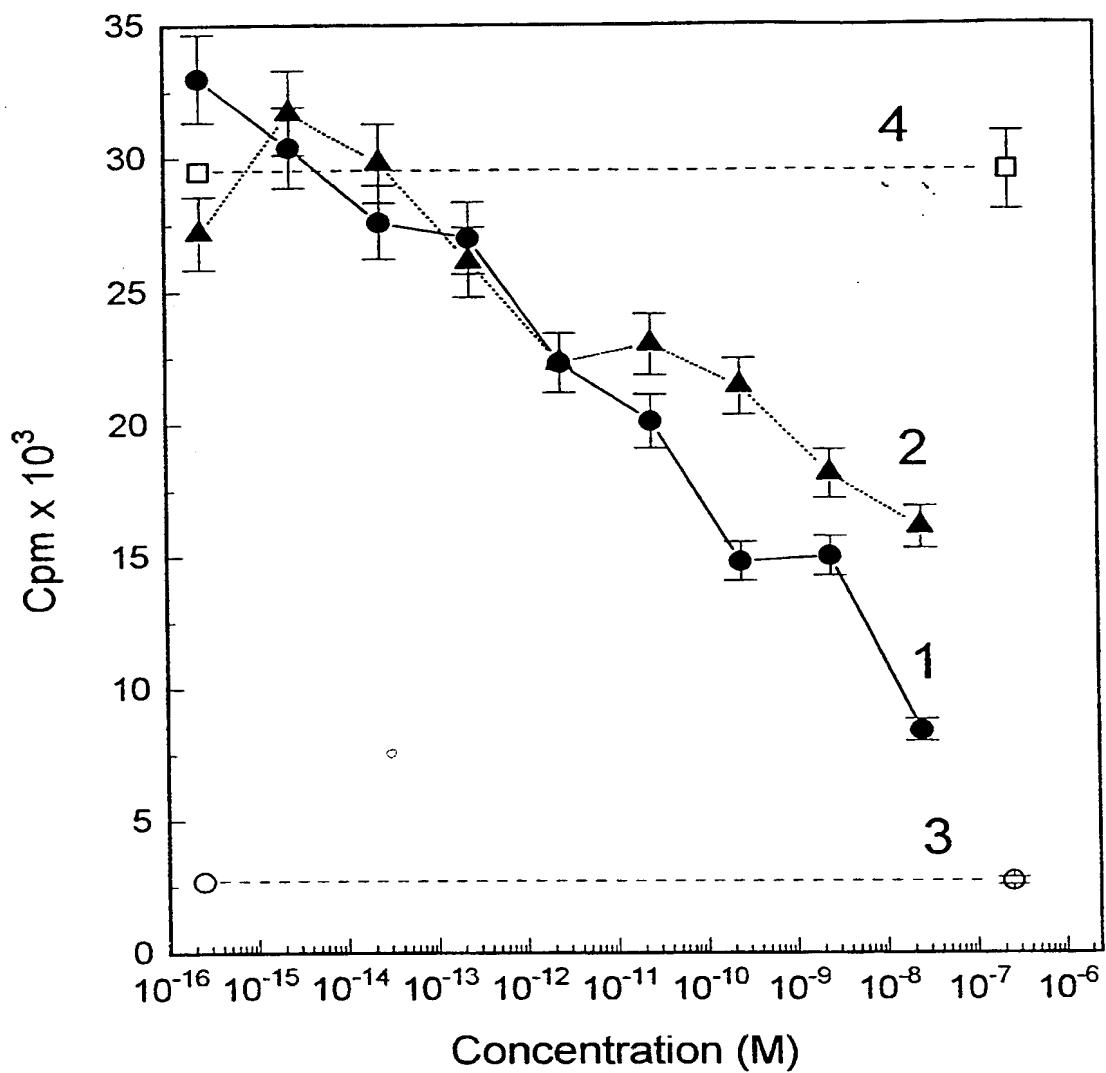


Fig.1

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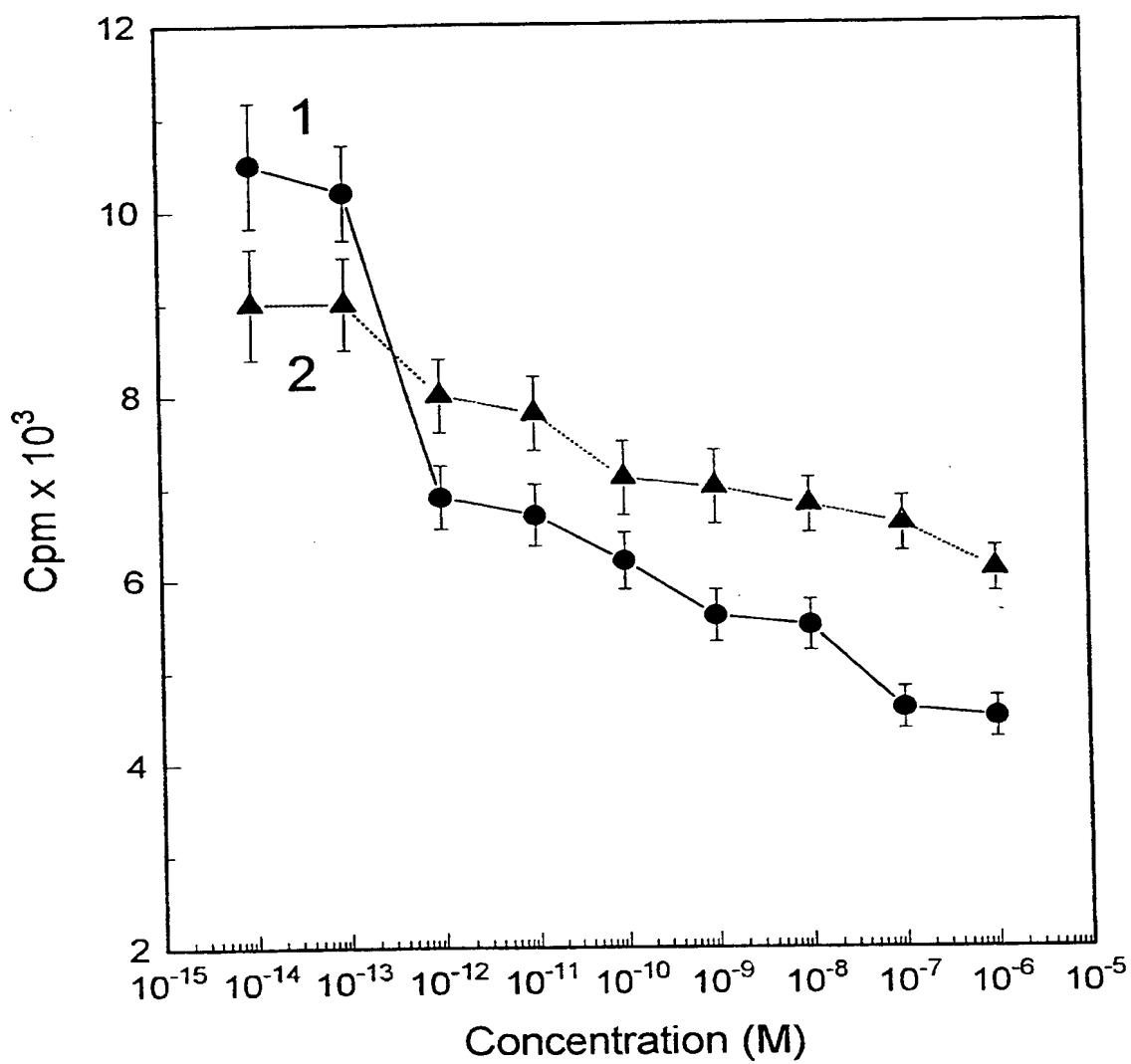


Fig.2

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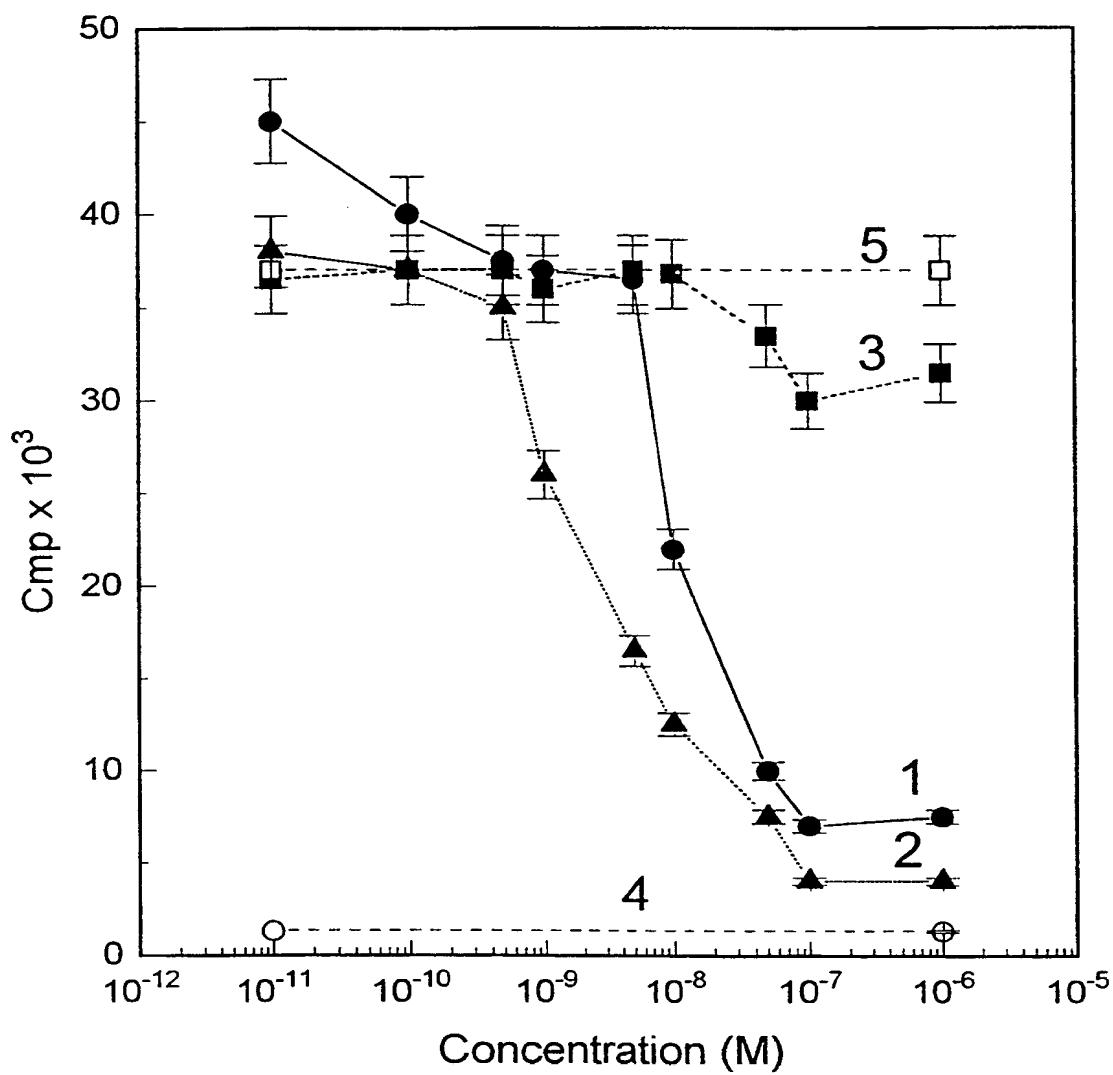


Fig. 3

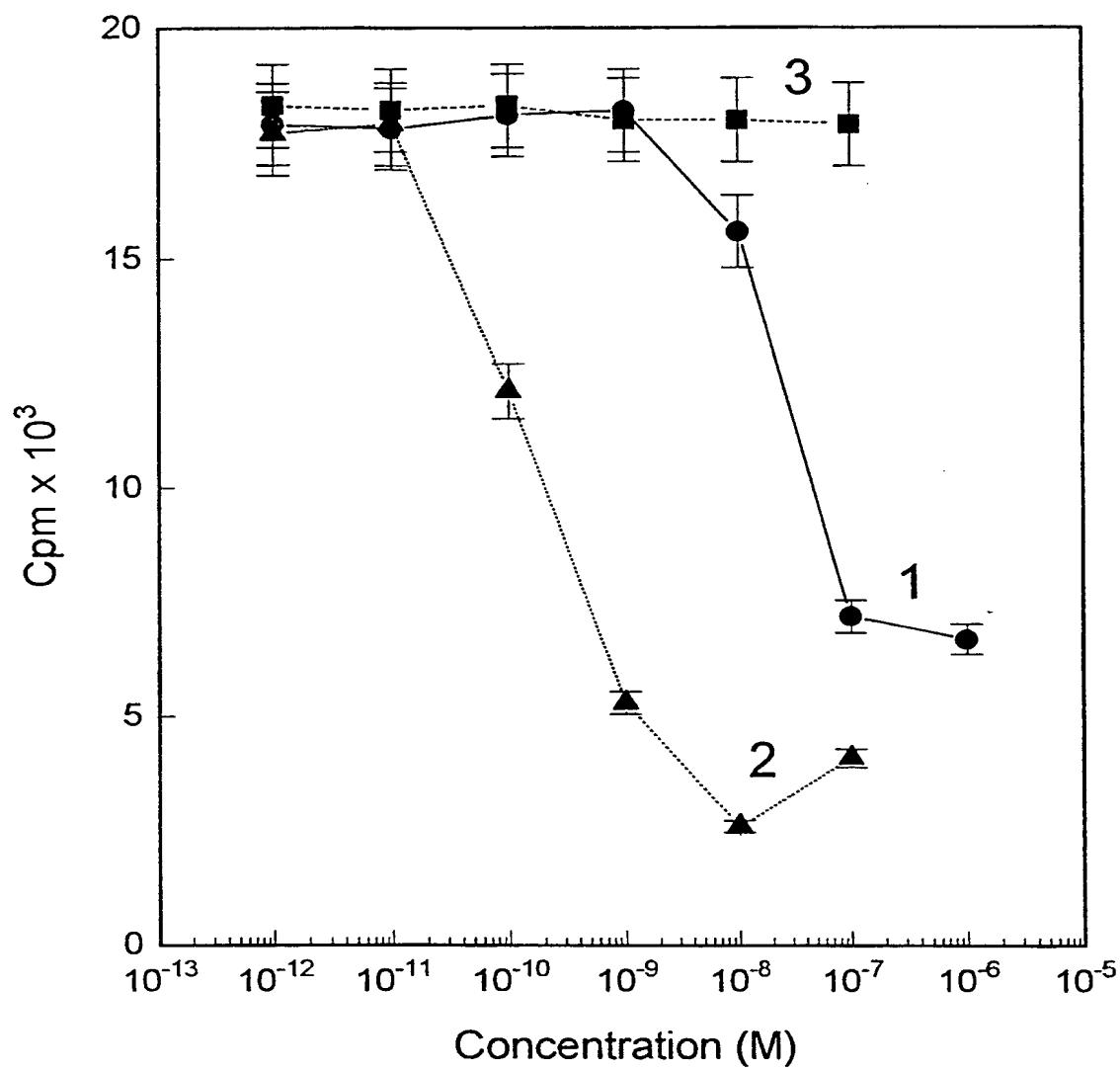


Fig.4

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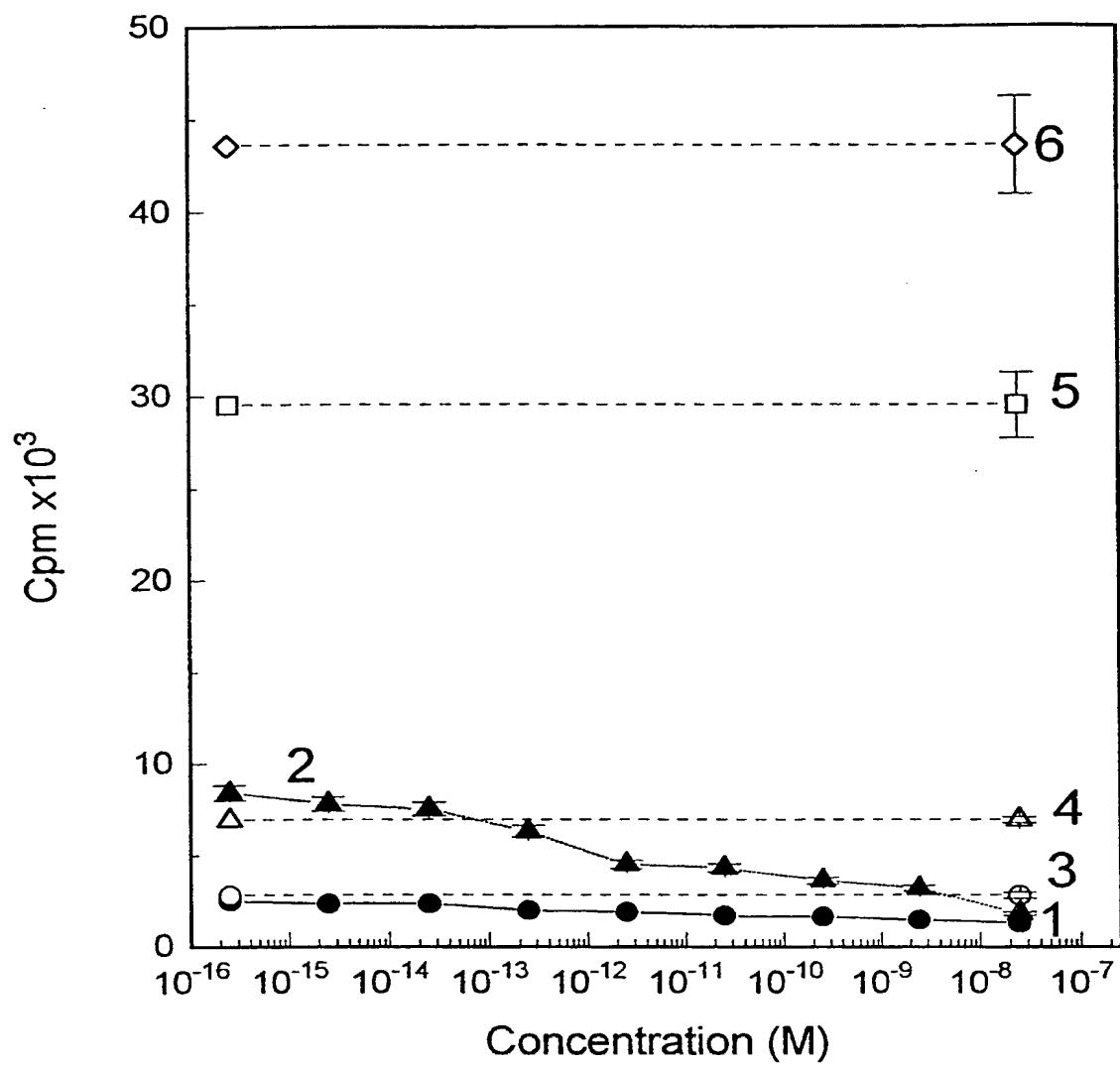


Fig. 5

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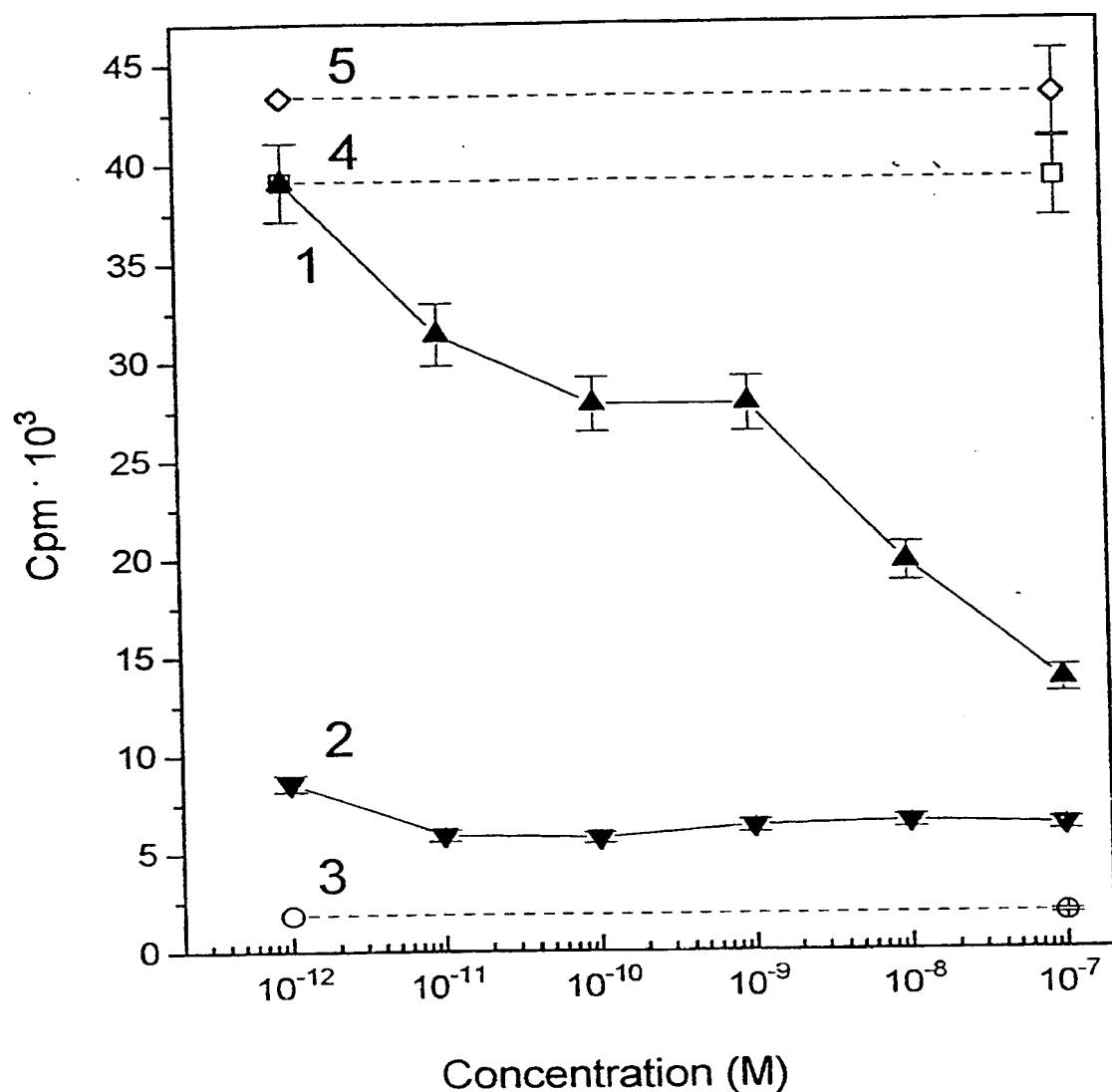


Fig. 6

INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 98/00418

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 38/04, A61K 38/13, A61K 38/21
 According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, REGISTRY, MEDLINE, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Dialog Information Services, file 155, Medline, Dialog accession no. 08309301, Medline accession no. 95272551, Zav'Yalov VP et al: "The sequence 130-137 of human interferon-alpha 2 is involved in the competition of interferon, prothymosin alpha and cholera toxin B subunit for common receptors on human fibroblasts"; & Mol Immunol, Apr 1995, 32 (6) p425-31 --	1-4
A	Dialog Information Services, file 73, Embase, Dialog accession no. 10073054, Zarogoulidis K. et al: "Interferon alpha-2a and combined chemotherapy as first line treatment in SCLC patients: A randomised trial"; & Lung Cancer, 1996, 15/2 (197-205) --	1-4



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

9 Sept 1998

10-09-1998

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 98/00418

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FR 2706772 A1 (VACSYN (S.A.)), 30 December 1994 (30.12.94), claims 1 and 12 --	1-4
A	WO 9410313 A2 (UNIVERSITY OF FLORIDA), 11 May 1994 (11.05.94), the abstract; page 30, line 27 - page 31, line 4; page 40, lines 27-33; page 41, lines 23-30 --	1-4
A	WO 9009806 A2 (UNIVERSITY OF FLORIDA), 7 Sept 1990 (07.09.90), page 3, lines 15-21; page 16, lines 15-19; and claim 17 -- -----	1-4

INTERNATIONAL SEARCH REPORT

Information on patent family members

27/07/98

International application No.

PCT/FI 98/00418

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
FR 2706772 A1	30/12/94	NONE		

WO 9410313 A2	11/05/94	AU 689450 B		02/04/98
		AU 5444994 A		24/05/94
		CN 1090510 A		10/08/94
		EP 0669981 A		06/09/95
		JP 8505047 T		04/06/96
		US 5705363 A		06/01/98
		US 5738845 A		14/04/98

WO 9009806 A2	07/09/90	AU 5183490 A		26/09/90
		US 5705363 A		06/01/98
		US 5738845 A		14/04/98



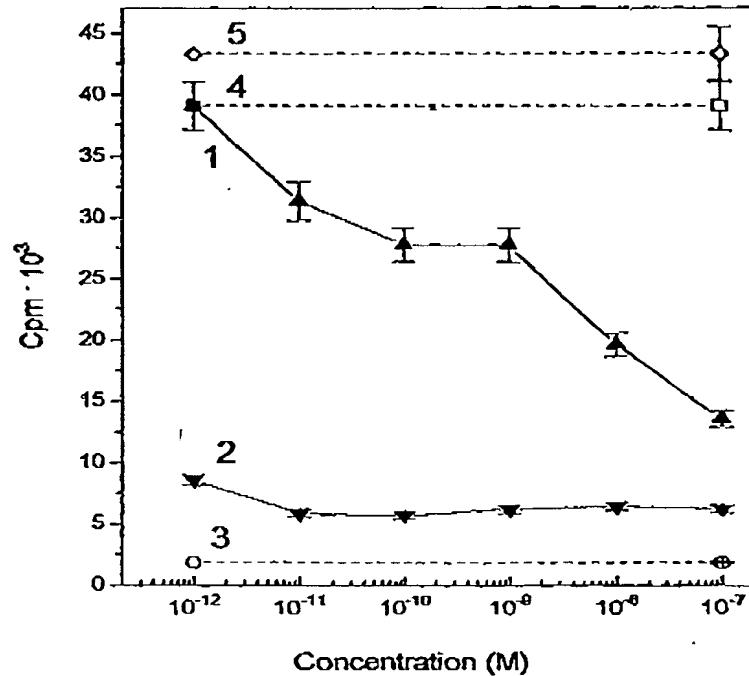
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 38/04, 38/13, 38/21		A1	(11) International Publication Number: WO 98/52594
			(43) International Publication Date: 26 November 1998 (26.11.98)
(21) International Application Number: PCT/FI98/00418		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 18 May 1998 (18.05.98)			
(30) Priority Data: 972121 19 May 1997 (19.05.97) FI			
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(54) Title: COMPOSITIONS FOR ENHANCING IMMUNOSUPPRESSANTS' PHARMACEUTICAL ACTIVITIES

(57) Abstract

The present invention provides the compositions for efficient amplification of immunosuppressive activity of cyclosporins, FK506 or rapamycin to decrease therapeutical dose of immunosuppressants, and as the consequence to avoid their undesirable side effects during organ and tissue transplantation, and treatment of different diseases. These compositions include cyclosporins, FK506 or rapamycin and biologically active peptides corresponding to the high-affinity binding/anti-lymphoproliferative site of IFNs- α , β , ω , τ , or recombinant proteins having the amino acid sequences corresponding to the said site.



INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 98/00418

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 38/04, A61K 38/13, A61K 38/21
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE, DK, FI, NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, REGISTRY, MEDLINE, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Dialog Information Services, file 155, Medline, Dialog accession no. 08309301, Medline accession no. 95272551, Zav'Yalov VP et al: "The sequence 130-137 of human interferon-alpha 2 is involved in the competition of interferon, prothymosin alpha and cholera toxin B subunit for common receptors on human fibroblasts"; & Mol Immunol, Apr 1995, 32 (6) p425-31 --	1-4
A	Dialog Information Services, file 73, Embase, Dialog accession no. 10073054, Zarogoulidis K. et al: "Interferon alpha-2a and combined chemotherapy as first line treatment in SCLC patients: A randomised trial"; & Lung Cancer, 1996, 15/2 (197-205) --	1-4

Further documents are listed in the continuation of Box C.

See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
9 Sept 1998	10-09-1998
Name and mailing address of the ISA Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carolina Palmcrantz Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

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		US 5705363 A		06/01/98
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